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MITOCHONDRIAL DNA POLYMORPHISM IN *DROSOPHILA ALBOMICANS* IN TAIWAN[1]

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Hwei-Yu Chang, Su-Hua Chang, Mei-Lien Chu and Fei-Jann Lin (1988) Mitochondrial DNA polymorphism in *Drosophila albomicans* in Taiwan. *Bull. Inst. Zool., Academia Sinica* 27(1): 27-35. *Drosophila albomicans* is one of the most abundant and widespread species in Taiwan. The well studied background information makes it a good candidate for studying population genetics and evolution. We have used two restriction endonucleases, EcoRI and HindIII, to analyze the mitochondrial DNA (mtDNA) of 359 isofemale stocks from 29 localities. Those restriction patterns reveal that mitochondrial DNA is polymorphic in local populations, but the number of major morphs is limited. There are two major EcoRI patterns, and two major HindIII patterns. But there are only two major composite EcoRI-HindIII patterns instead of four. Different morphs may have different selection values, in other words the variation of restriction pattern is probably not neutral. Although the mitochondrial DNA is polymorphic within species, the hypothetical primitive morph may still be useful information source for reconstruction phylogeny among closely related species.

Key words: *Drosophila*, mtDNA, Polymorphism.

In the past two decades, molecular data are frequently used to reveal relationships among populations. Compairing to the information acquired from proteins, information from DNA is more direct estimates of genetic variation. Animal mitochondrial DNA, which evolves five to ten times more rapidly than single copy nuclear DNA in vertebrates (Brown and Wright, 1979; Brown, *et al.*, 1982; Vawter and Brown, 1986), is a handy tool to study relationships among populations as well as that among closely related species (Avise, *et al.*, 1979a, 1979b, 1986; Ferris, *et al.*, 1981, 1983; Shah and Langley, 1979; Yonekawa, *et al.*, 1981; Templeton, 1983).

Drosophila albomicans is a member of *D. nasuta* subgroup which belongs to *D. immigrans* species group. *D. nasuta* consists of about ten closely related species. Before we can use mtDNA data to study the phylogeny of those species, we have to know whether there is intraspecific variation. The purposes of the study are to survey restriction patterns of the mitochondrial DNA of the *Drosophila albomicans* in order to reveal the intra- and inter-population variation, and to find out whether a collection of isofemale stocks maintained in the laboratory can represent the natual population.

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MATERIALS AND METHODS

Flies

One hundred and fifty three isofemale stocks from 29 localities (Fig. 1) in Taiwan were maintained in the laboratory for about ten years, and two hundred and six isofemale stocks from 15 localities were newly established during the year 1985.

Extraction of mitochondrial DNA

Mitochondrial DNA was extracted from larvae according to the protocol modified from that reported by Coen, *et al.* (1982). Briefly, 15 larvae were gently homogenated in 320 μl of solution I (10 mM Tris, pH 7.8, 60 mM NaCl, 5% sucrose, and 10mM EDTA), and lysed by the addition of 400 μl of freshly mixed solution II (300 mM Tris, pH 9.0, 1.25% SDS, 5% sucrose, 100 mM EDTA, and 8 μl/ml diethylpyrocarbonate). After the reaction mixtues were incubated at 65°C for

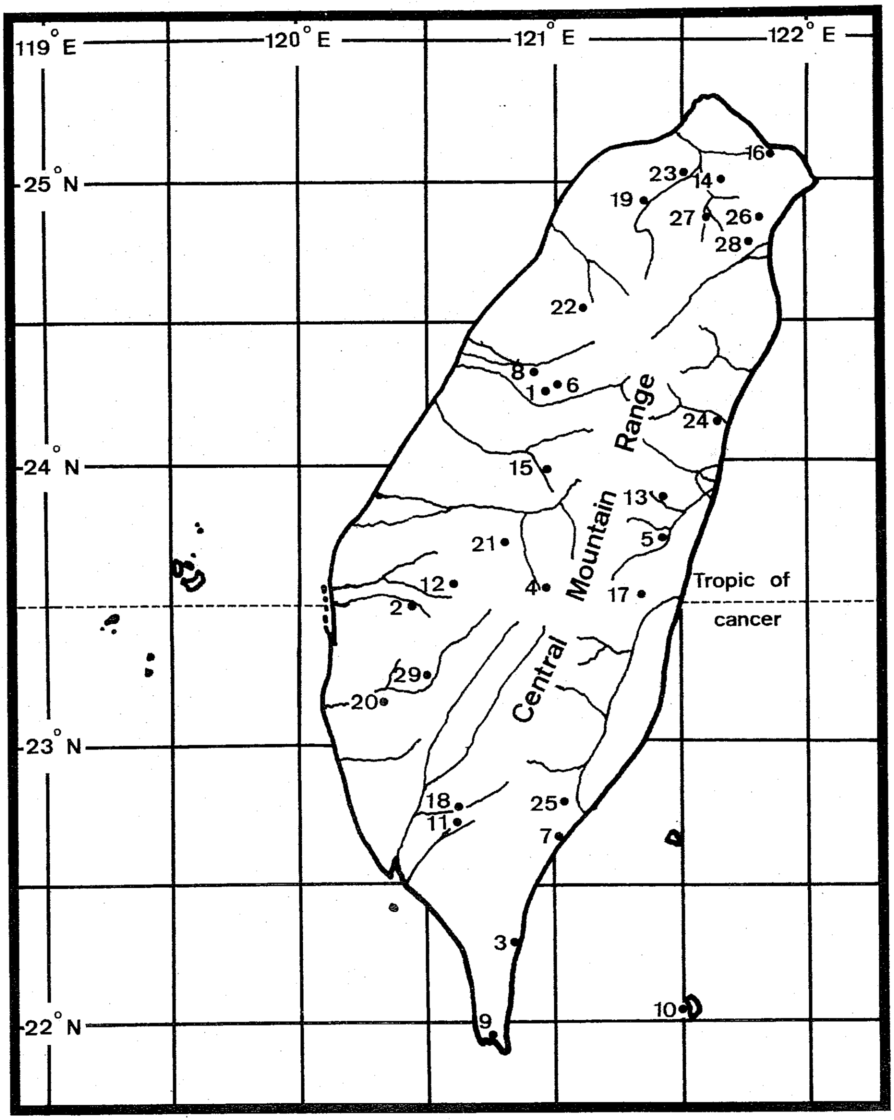

Fig. 1. Twenty nine collecting sites of *Drosophila albomicans* are indicated on the map of Taiwan. See Table 3 for the names of these localities.

30 min, 120 μl of 3 M sodium acetate was added. Stand on ice for 45 min, and then centrifuge on Eppendorf for 10 min at 4°C. DNA was precipitated by isopropanol at room temperature, and resuspended in 250 μl double distilled water containing 0.6 μl diethylpyrocarbonate (freshly mixed). Stand at room temperature for 20 min, and then add another 250 μl double distilled water. Mitochondrial DNA was precipitated by ethanol and dried under vaccum.

Digestion of restriction endonucleases and agarose gel electrophoresis

Mitochondrial DNA, extracted by the method described above, was dissolved in 20 μl double distilled water. The digestion of restriction endonucleases was performed according to the procedure described by Maniatis, *et al.* (1982). The reaction was stopped by the addition of 1/10 volumn STOP solution (0.05% bromophenol blue, 50 mM EDTA, 0.05% SDS, 50% glycerol, and 2 mg/ml boiled RNase). The restriction pattern was analyzed on a 0.8% agarose gel in Tris-acetate buffer (4 mM Tris, pH 8.1, 2 mM acetic acid, and 0.2 mM EDTA). After staining with ethidium bromide, DNA bands were visualized under UV light. Size of fragments were estimated by a graphical method using HindIII digested lambda phage DNA as size standard.

RESULTS

MtDNA from 359 isofemale stocks was subjected for HindIII and EcoRI digestion. Since the enzyme digestion was done by using crude extract of mtDNA, individual results on gel are not good representatives of restriction patterns. Besides, the DNA fragments ranging from 11.8 kb to 1.1 kb do not appear clearly on a same gel (Fig. 2). We use the average of about 30 measurement data for each restriction pattern to produce the graph of Fig. 3. Size determination was performed by running mtDNA restriction fragments, mtDNA restriction fragments+DNA size standard, and DNA size standard side-by-side on a gel. Size of large fragments was determined on low concentration agarose gel, and that of small fragments on high concentration gel. There are 7 restrition

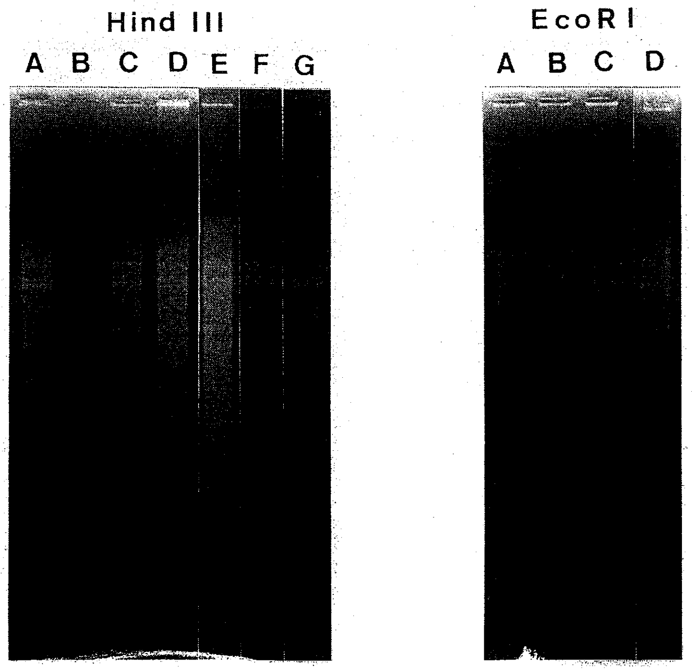

Fig. 2. Photo of seven HindIII restriction patterns and 4 EcoRI restriction patterns of mitochondrial DNA.

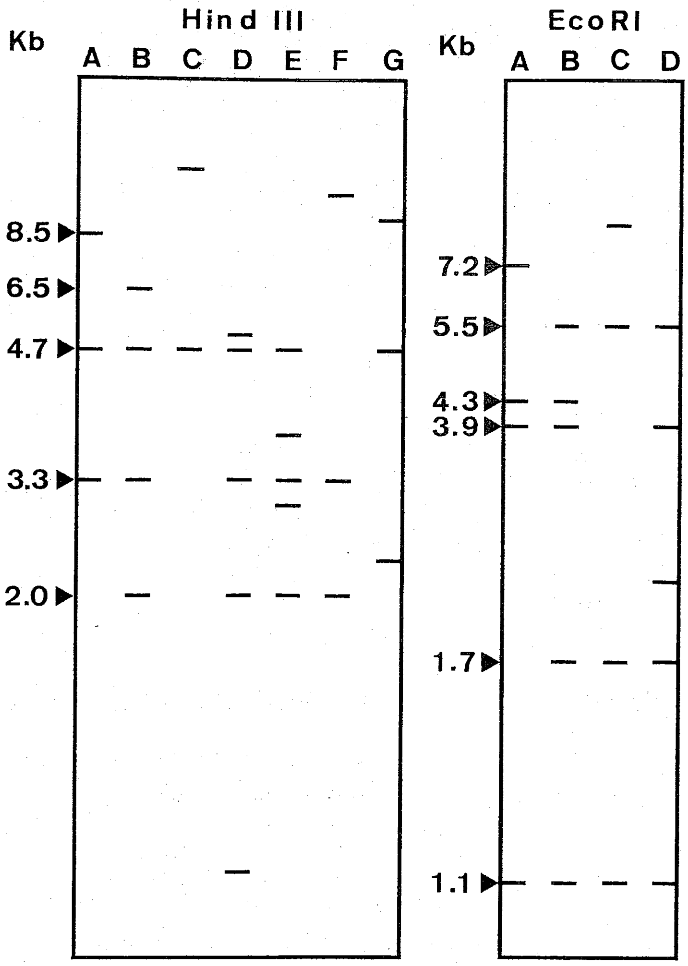

Fig. 3. Restriction patterns are showed graphically with sizes of fragments indicated beside.

patterns (A, 61.0%; B, 35.3%; C, 1.1%; D, 0.6%; E, 0.3%; F, 0.3%; and G, 1.4%) resulted from the digestion with HindIII, and 4 (A, 30.6%; B, 66.1%; C, 2.5%; and D, 0.8%) with EcoRI (Fig. 3). If the mtDNA in *D. albomicans* evolves from the same ancestor, we may construct a network to connect these restriction patterns. These restriction patterns have been arranged in Fig. 4 so as to minimize the number of restriction sites that need to be gained or lost in network. In all cases the transition from one pattern to another requires just a single-site change (gain or loss). As shown in Fig. 4, these two restriction endonucleases, HindIII and EcoRI, yield a total of 13 mtDNA restriction sites in the 359 isofemale stocks; 4 sites (31%) are shared by all stocks, the other 9 (69%) are polymorphic. Pattern A of HindIII digestion (219 stocks) and pattern B of EcoRI digestion (237 stocks) are the main patterns found in *D. albomicans* on this island.

All of the composite patterns (morphs) found are showed in Table 1. Only 14 out of 28 hypothetical morphs are observed in this study. The frequency distribution of those morphs does not fit the expected frequency calculated from single digestion data (Table 1). Two mtDNA morphs, 2 and 4, are common. Morph 2 is presented in 205 (57.1%) and morph 4 in 92 (52.6%) of the 359 *D.*

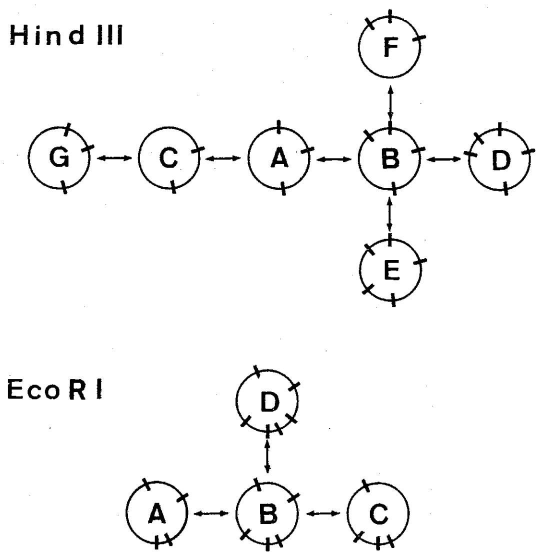

Fig. 4. Diagram of the restriction patterns obtained with endonucleases HindIII and EcoRI in *D. albomicans* mtDNA. The most common restriction patterns are HindIII A and EcoRI B. Locations shown for the restriction sites are arbitrary.

albomicans isofemale stocks. The remaining twelve morphs consist of just 17.3% of the population. Six of them are found in single stocks, and the other six are scatteredly distributed.

Table 2 shows the distribution of mtDNA morphs in old laboratory maintained and newly established isofemale stocks. According to χ^2 test, there is no significant difference between these two groups. So the data of old stocks and that of new stocks are combined and the result of this survey is summarized in Table 3. MtDNA seems to be polymorphic in local populations, and to

TABLE 1

Restriction pattern for the 14 mtDNA morphs found in *D. albomicans*

Morph	Restriction pattern HindIII	Restriction pattern EcoRI	Stocks Number	Stocks Percent	Expected percent
1	A	A	13	3.6	18.67
2	A	B	205	57.1	40.32
3	A	C	1	0.3	1.52
4	B	A	92	25.6	10.80
5	B	B	24	6.7	23.33
6	B	C	8	2.2	0.89
7	B	D	3	0.8	0.28
8	C	A	3	0.8	0.34
9	C	B	1	0.3	0.73
10	D	A	1	0.3	0.18
11	D	B	1	0.3	0.40
12	E	A	1	0.3	0.09
13	F	B	1	0.3	0.20
14	G	B	5	1.4	0.92

TABLE 2
The distribution of mtDNA morphs in old laboratory maintained and newly established isofemale stocks of *D. albomicans*

	Morphs													
Stocks	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Old	5	90	1	41	8	1	2	0	0	1	1	1	0	2
New	8	115	0	51	16	7	1	3	1	0	0	0	1	3
Total	13	205	1	92	24	8	3	3	1	1	1	1	1	5

TABLE 3
The distribution of mtDNA morphs in *D. albomicans*

	No. of	Morphs													
Locality	stock	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Baileng (白冷)	16		11		3						1				1
2. Chia-yi (嘉義)	27	1	11		8	3	3		1						
3. Dawu (大武)	4		3			1									
4. Dungpu (東埔)	7		5			2									
5. Funglin (鳳林)	17		10		4	2				1					
6. Guguan (谷關)	5		4		1										
7. Jrben (知本)	3		2					1							
8. Juolan (卓蘭)	17	1	9		4		3								
9. Kending (墾丁)	42	1	22		12	4	1		2						
10. Lanyu (蘭嶼)	2		1		1										
11. Majia (瑪家)	1		1												
12. Meishan (梅山)	12	1	6		4								1		
13. Muguashan (木瓜山)	6	1	5												
14. Nan-gang (南港)	5		3		2										
15. Puli (埔里)	17		7		6	3						1			
16. Rueifang (瑞芳)	4		3		1										
17. Rueisuei (瑞穗)	18		10		6	1	1								
18. Sandimen (三地門)	4	1	2		1										
19. Sanshia (三峽)	4	1	3												
20. Shanhutan (珊瑚潭)	7		6		1										
21. Shitou (溪頭)	9		9												
22. Shrtoushan (獅頭山)	10	1	4		3	2									
23. Taibei (臺北)	1		1												
24. Tailuge (太魯閣)	24		12		9	2									1
25. Taiping (太平)	1					1									
26. Wufeng (五峯)	1				1										
27. Wulai (烏來)	48		27	1	17	1		1							1
28. Yilan (宜蘭)	1														1
29. Yuenshuei (沄水)	46	5	28		8	2		1						1	1

have a couple of common major morphs in all populations.

DISCUSSION

The size of the *D. albomicans* mtDNA is estimated to be 16.5 kilobases (kb), which is about average for animals. The range for most animals studied is 15.7 to 19.5 kb (Brown, 1983). No length polymorphism was observed among the 359 isofemale stocks. In the strict sense, no size difference which is larger than 0.5 kb has been found in this study, but the restriction digestion shows polymorphism. The laboratory maintained isofemale stocks are still good representatives to natural population on the mtDNA variation.

Five localities, each represented by only one stock, do not provide useful data for analysis of local population variation. All the remaining populations, execpt the one at Shitou including 4 new stocks and 5 old stocks, are shown to be polymorphic. According to our observation, even for the flies collected from a single bait there usually exists polymorphism. One possible explanation for the monomorphism in Shitou is that this local population has been maintained in small size and is insulated, since the possibility of having the same morph in 9 collections from 1971 to 1985 is low. Genetic dirft plus the inhibition of migration may be the cause of monomorphism.

The fact of the high polymorphism in mtDNA as well as the scattered distribution of minor morphs in populations is conflicting to the common thought that mtDNA is homogenous within a small population because of its clonal inheritance (Brown, 1983). This result may be partly explained by a high mutation rate of mtDNA. Therefore, the presence of a certain restriction site may be the result of convergent or parallel evolution instead of common ancestry of populations. This phenomenon is quite different from chromosomal inversion data. Because of the extremely low mutation rate of variable inversions, a population loses or gains a chromosomal inversion through genetic drift and migration, respectively. Thus, the presence of a particular chromosomal inversion type instead of a particular mtDNA restriction site may reveal geneology of populations.

The analysis of nucleotide substitutions between pairs of DNA sequences can not directly reveal the phylogenetic relationship of species simply because of polymorphism. This problem may be solved by increasing sample size of each population and by using a large number of restriction enzymes as recommended by Nei and Taijma (1983). However, the phylogenetic tree of populations constructed from the frequency distribution data of restriction patterns may still be screwed up by selection and nonrandom mutation, even if the sample size is large enough. By using a large number of restriction enzymes may help us minimize the bias resulted from selection and nonrandom mutation.

The other alternative is that the phylogenetic relationship of species may be reconstructed by the primitive morphs of mtDNA. How to recognize the primitive morph will be a difficulty in this aspect. In our case, we consider HindIII pattern B and EcoRI pattern B to be the primitive forms (Fig. 4), then morph 5 (Table 1) should be the primitive composite form. If this is true, the most abundant form is not the most primitive form. The two major morphs in the present populations are both derived from this hypothetical primitive form. This result may also suggest that the hypothetically primitive form is not the fittest on this island. The fact that all of those large samples have the same major morph in spite of the population differentiation showed by our previous study (Lin and Chang, 1986) indicates this major morph really have advantage on fitness in Taiwan.

In this study, there are six morphs which appear in single stocks. The other six minor morphs ranging from three to 24 stocks show scattered distribution. In *D. subobscura*, however, six minor morphs appear to be narrow endemic, each found in only one locality (Latorre, *et al.*, 1986). This inconsistancy may be due to sample size. Five out of the six minor *D. subobscura* morphs were found in single strains. And the only informative morph appears in just two strains.

In conclusion: (1) mtDNA is polymorphic within local populations; (2) mtDNA does not show strong differentiation among populations; (3) there are two major morphs presented in *D. albomicans* on this island; and (4) neither of the two major morphs is the hypothetical primitive morph.

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REFERENCES

Avise, J. C., R. A. Lansman and R. O. Shade (1979a) The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. *Genetics* **92**: 279-295.

Avise, J. C., C. Giblin-Davidson, J. Laerm, J. C. Patton and R. A. Lasman (1979b) Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. *Proc. Natl. Acad. Sci. U. S. A.* **76**: 6694-6698.

Avise, J. C., G. S. Helfman, N. C. Saunders and L. S. Hales (1986) Mitochondrial DNA differentiation in North Atlantic eels: Population genetics consequences of an unusual life history pattern. *Proc. Natl. Acad. Sci. U. S. A.* **83**: 4350-4354.

Brown, W. M. (1983) Evolution of animal mitochondrial DNA. In *Evolution of Genes and Proteins* (M. Nei and R. K. Koehn, eds.). Sinauer Associates Inc., Massachusetts. pp. 62-88.

Brown, W. M. and J. M. Wright (1979) Mitochondrial DNA analyses and the origin and relative age of parthenogenetic lizard (genus *Cnemidophorus*). *Science* **203**: 1247-1249.

Brown, W. M., E. M. Prager, A. Wang and A. C. Wilson (1982) Mitochondrial DNA sequences of primates: Tempo and mode of evolution. *J. Mol. Evol.* **18**: 225-239.

Coen, E. S., J. M. Thoday and G. Dover (1982) Rate of turnover of structural variants in the rDNA gene family of *Drosophila melanogaster*. *Nature* **295**: 564-568.

Ferris, S. D., A. C. Wilson and W. M. Brown (1981) Evolutionary tree for apes and humans based on cleavage maps of mitochondrial DNA. *Proc. Natl. Acad. Sci. U. S. A.* **78**: 2432-2436.

Ferris, S. D., R. D. Sage, C. M. Huang, J. T. Nielsen, U. Ritte and A. C. Wilson (1983) Flow of mitochondrial DNA across a species boundry. *Proc. Natl. Acad. Sci. U. S. A.* **80**: 2290-2294.

Latorre, A., A. Moya and F. J. Ayala (1986) Evolution of mitochondrial DNA in *Drosophila subobscura*. *Proc. Natl. Acad. Sci. U. S. A.* **83**: 8649-8653.

Lin, F. J. and H. Y. Chang (1986) Chromosomal inversions in *Drosophila albomicans* in Taiwan. *Bull. Inst. Zool., Academia Sinica* **25**: 129-134.

Maniatis, T., E. F. Fritsch and J. Sambrool (1982) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, New York. 545 pp.

Nei, M. and F. Tajima (1983) Maximum likelihood estimation of the number of nucleotide substitutions from restriction site data. *Genetics* **105**: 207-217.

Shah, D. M. and V. D. Bergh (1979) Inter- and intra-specific variation in restriction maps of *Drosophila* mitochondrial DNAs. *Nature* **281**: 696-699.

Templeton, A. R. (1983) Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37**: 221-244.

Vawter, L. and W. M. Brown (1986) Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. *Science* **234**: 194-196.

Yonekawa, H., K. Moriwaki, O. Gotoh, J. I. Hayashi, J. Watanabe, N. Miyashita, M. L. Petras and Y. Tagashira (1981) Evolutionary relationships among five subspecies of *Mus musculus* based on restriction enzyme cleavage patterns of mitochondrial DNA. *Genetics* **98**: 801-816.

紅果蠅 (*Drosophila albomicans*) 的臺灣族羣之粒腺體DNA多態性

張慧羽　張素華　朱美蓮　林飛棧

紅果蠅（*Drosophila albomicans*）在臺灣數量多且分佈廣，而且已有許多研究資料，因此很適宜用來研究族羣遺傳與進化。我們採用兩種限制酶 EcoRI 和 HindIII 來分析採自臺灣29個地區的359個單雌品系之粒腺體 DNA。結果顯示卽使在一個小區域裏，粒腺體 DNA 仍然呈多態性。EcoRI 限制酶切出兩種主要的型式，HindIII 亦然，而 EcoRI-HindIII 的組合限制酶切型却只有兩種主要的型式，限制酶切型或許會造成個體間選汰上的差異，換言之，限制酶切型的變異或許並非中性變異，雖然種內粒腺體 DNA 具多態性，但假設的原始限制酶切型對於近緣種的種間關係之建立仍可提供有用的資料。